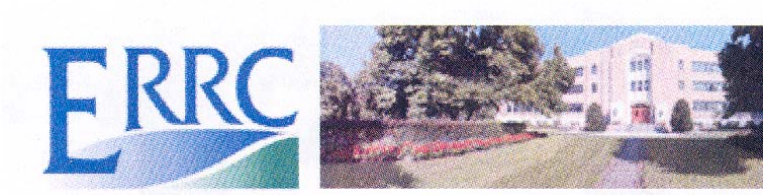

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Title: Quality of Alfalfa Sprouts Grown from Irradiated Seeds

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QUALITY OF ALFALFA SPROUTS GROWN FROM IRRADIATED SEEDS[1]

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ABSTRACT

Sprouts grown from nonirradiated alfalfa seeds and those irradiated at 1, 2, and 3 kGy were stored at 7C for 21 days. Sprout quality was measured initially and after 21 days storage. Compared with nonirradiated controls, sprouts grown from irradiated seeds had an increased vitamin C content and antioxidant activity measured by the ferric reducing antioxidant power (FRAP) assay both initially and after 21 days. The vitamin C, chlorophyll and carotenoid contents as well as antioxidant activity all decreased during storage. Levels of chlorophyll and carotenoid were similar in sprouts grown from irradiated and nonirradiated seeds both initially and after storage. Sprouts from irradiated seeds also had short roots compared with those from nonirradiated seeds.

INTRODUCTION

Outbreaks of foodborne illness have been associated with consumption of seed sprouts (Taormina *et al.* 1999). The outbreaks have been due to the presence of a large number of *Escherichia coli* O157:H7 and *Salmonella* spp. in sprouts grown from contaminated seeds. Mild temperature (20-24C) and a high moisture environment used for sprout production favor the multiplication of bacteria (Jaquette *et al.* 1996). Treatment of seeds with sanitizers has not always proven effective in eliminating pathogens (Weissinger and Beuchat 2000), in part, due to possible internalization of pathogens (Moline and Kulik 1997). The process of drying seeds after contamination protects pathogens that

[1] Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture above others of similar nature not mentioned.

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have entered the seeds through cracks and crevices against inactivation by chlorine or other chemical treatments (Jaquette *et al.* 1996). Furthermore, the high level of organic matter associated with seed germination diminishes the efficacy of many treatments unless concentrations are used which seriously reduce germination rate (Jaquette *et al.* 1996). The internalization and the rapid growth of bacteria during sprouting make many chemical treatments impossible to warrant a safe final product — sprouts. It has been shown that irradiation effectively inactivated *E. coli* O157:H7 and *Salmonella* spp. from both seeds and sprouts (Rajkowski and Thayer 2000, 2001). The FDA has approved the use of ionizing radiation at doses up to 8 kGy to control microbial pathogens in seeds for sprouting (FDA 2000). The nutritional value of alfalfa sprouts from irradiated seeds is not clear.

Alfalfa sprouts are a nutritious food and contains many antioxidants, including vitamin C and carotenoids (Augustin *et al.* 1983; Hamilton and Vanderstoep 1979). The antioxidant content of alfalfa sprouts is one of the highest among common vegetables (Cao *et al.* 1996). In our earlier report, we have shown that irradiation of sprouts at doses up to 2.6 kGy did not negatively impact quality of sprouts, and in fact, irradiated sprouts had higher antioxidant power than nonirradiated ones (Fan and Thayer 2001). In the present report, we have studied the quality of sprouts raised from irradiated seeds.

MATERIALS AND METHODS

Alfalfa seeds obtained from a commercial seed supplier were irradiated at 0, 1, 2 and 3 kGy using a gamma source and sprouted in a commercial facility (Snider's Sprouts, Potomac, MD). The time between irradiation and beginning germination was less than 1 week. The seeds were sanitized with 20,000 ppm calcium hypochlorite, rinsed with water, placed in a rotating drum for 24 h, and placed onto trays which were then transferred into the growth chambers. During sprouting, water was sprayed for 1 min every 30 min. Sprouts were harvested 4 days later. A detailed description of irradiation and growing of sprouts was reported earlier (Rajkowski and Thayer 2001). After harvest, the sprouts were transported to our research center in an air-conditioned van and stored at 7C in plastic pans covered with aluminum foil. There was no light exposure during the entire storage period. Color, soluble solids content, antioxidant activity, ascorbic acid and dehydroascorbic acid were measured 1 and 21 days after harvest using methods described by Fan and Thayer (2001). There were four replicates per dose. The shoot and root lengths were measured using a ruler at day 1. Four 1 g sprouts were used for the length measurements for each dose.

Respiration

Respiration was measured at 1, 8 and 21 days of storage. Fifty gram samples were enclosed in 490 mL glass jars and sealed for 10-12 h at 7C, then 0.5 mL headspace was withdrawn from the jars through septa, and injected into an HP5890 gas-chromatograph (Agilent Technologies Inc., Palo Alto, CA) equipped with a 183 cm CTR I column (Alltech Associates, Inc., Deerfield, Ill.) and a thermal conductivity detector. The CTR I column consists of an outer column (0.64 cm i.d) packed with an activated molecular sieve and an inner column (0.32 cm i.d.) packed with a porous polymer mixture. The injector, oven, and detector temperatures were held at 150, 30 and 150C, respectively. The carrier gas was helium at a flow rate of 65 $mL \cdot min^{-1}$. CO_2 was calculated in comparison to a standard.

Vitamin C Measurement

Analysis of ascorbic acid and vitamin C (ascorbic acid plus dehydroascorbic acid) was measured using a high-performance liquid chromatograph according to Graham and Annette (Graham and Annette 1992) with minor modifications. A detailed description of the extraction and analysis was reported earlier (Fan and Thayer 2001).

Analysis of Antioxidant Power

Samples (10 g) were extracted with 15 mL cold ethanol using a homogenizer (Virtishear, Virtis, Gardiner, NY) at a speed setting of 70 for 2 min. The homogenate was filtered through a glass fiber filter (GF/A, Whatman, Clifton, NJ) under vacuum, and the filtrate was transferred into a 25 mL volumetric flask and filled to volume using ethanol. Antioxidant power in the crude extract was determined using the ferric reducing antioxidant power (FRAP) assay (Benzie and Strain 1996). The FRAP reagent was prepared daily by combining 300 mM acetate buffer (pH 3.6), 10 mM 2,4,5-tripyridyl-s-triazine in 40 mM HCl, and 20 mM $FeCl_3$ in the ratio of 10:1:1 (v:v:v). Three milliliters of FRAP reagent were added to 100 μL extract. The mixture was incubated at 23C for 30 min before absorbance at 593 nm was measured with a Sargent-Welch 6-550 UV/VIS spectrophotometer (Pye Unicam Ltd., Cambridge, UK). FRAP values were calculated from an ascorbic acid standard curve.

Measurement of Carotenoid and Chlorophyll

The pigments were extracted with cold acetone and then partitioned into diethyl ether. Pigments in diethyl ether were measured with a spectrophotometer. A more detailed description of the procedure was reported earlier (Fan and Thayer 2001).

Statistical Analysis

There were four replicates for each quality parameter. Data were subjected to statistical analysis using SAS ver. 6.12 (SAS Institute, Cary, NC). Various quality parameters as a function of radiation dose and storage time were analyzed by the least significant difference (LSD) analysis using the general linear model (GLM) procedure.

RESULTS AND DISCUSSION

Sprouts from irradiated seeds had higher respiration rates than those from nonirradiated seeds at 1 and 8 days after harvest. But the difference in respiration rate disappeared at 21 days (Table 1). The irradiation-induced increase in respiration has been documented in various plant tissues (Maxie and Abdel-Kader 1966). Our results indicate that sprouts raised from irradiated seeds also had higher respiration rates. The high respiration of sprouts from irradiated seeds may indicate the seeds had been stressed or injured by irradiation.

TABLE 1.
RESPIRATION RATE (ML $CO_2 \cdot H^{-1} \cdot KG^{-1}$ FRESH WEIGHT) OF ALFALFA SPROUTS DURING STORAGE AT 7C

Radiation dose (kGy)	Storage time (day)		
	1	8	21
0	37.6 ±0.4[b]	29.3 ±0.8	30.7 ±1.8
1	40.4 ±1.1	32.4 ±0.7	29.7 ±0.5
2	39.9 ±1.6	32.6 ±1.7	30.6 ±1.8
3	39.8 ±1.2	31.8 ±1.0	30.6 ±0.9
$LSD_{0.05}$[a]	1.8	1.7	2.1

The sprouts were raised from seeds irradiated with 0, 1, 2, and 3 kGy gamma radiation. The sprouts were then stored at 7C for 21 days. Respiration was measured at 1, 8, and 21 days after harvest.
[a] The least significant difference at $P < 0.05$.
[b] The values are means of four replicates followed by standard deviations.

Sprouts grown from all irradiated seeds had greater antioxidant power than those from nonirradiated seeds at both 1 and 21 days after harvest (Table 2).

Overall, antioxidant power of all sprouts decreased during storage. It has been demonstrated that seed irradiation, depending on dose, can either increase or decrease antioxidant activity of wheat, corn and buckwheat seedlings (Gorlanov and Kokorev 1973). Our results indicate that alfalfa sprouts from seeds irradiated in the dose range of 1-3 kGy always had higher antioxidant activity than those grown from nonirradiated seeds. There are many antioxidants in plant tissues, and one of the most common antioxidants in fresh fruits and vegetables is ascorbic acid.

TABLE 2.
TOTAL ANTIOXIDANT POWERS (μMOL·G^{-1} FRESH WEIGHT) OF SPROUTS DURING STORAGE AT 7C

Radiation dose (kGy)	Storage time (day)		
	1	21	
	Total antioxidant power		
0	2.2±0.1[c]	1.7±0.2	*[b]
1	2.7±0.4	2.3±0.1	NS
2	2.8±0.1	2.5±0.1	*
3	2.9±0.2	2.4±0.2	*
$LSD_{0.05}$[a]	0.4	0.2	

The sprouts were raised from seeds irradiated with 0, 1, 2, and 3 kGy gamma radiation. Antioxidant power was measured at 1 and 21 days after harvest.

[a] The least significant difference at $P < 0.05$.

[b] NS, *, or ** indicates nonsignificant or significant at $P < 0.05$, and 0.01, respectively.

[c] The values are means of four replicates followed by standard deviations.

Sprouts grown from irradiated seeds had higher vitamin C content than those grown from nonirradiated seeds 1 day after harvest (Table 3). The increase in vitamin C content was directly related to the absorbed radiation dose. After 21 days storage, vitamin C content of sprouts raised from irradiated seeds remained higher than that from nonirradiated seeds. Sprouts raised from seeds irradiated at 3 kGy had more than twice the amount of vitamin C than those raised from nonirradiated seeds at day 21. Independent of radiation dose, Vitamin C content decreased during storage. The decrease in vitamin C content during storage has been observed in alfalfa sprouts (Fan and Thayer 2001).

TABLE 3.
VITAMIN C CONTENT ($\mu G \cdot G^{-1}$ FRESH WEIGHT) OF SPROUTS DURING STORAGE AT 7C

Radiation dose (kGy)	Storage time (day)		
	1	21	
0	61.8±2.9[c]	25.1±9.2	*[b]
1	90.6±12.3	45.4±12.6	*
2	92.7±9.1	46.7±8.2	*
3	103.9±11.8	53.1±9.3	*
$LSD_{0.05}$[a]	13.9	11.8	

The sprouts were raised from seeds irradiated with 0, 1, 2, and 3 kGy gamma radiation. Vitamin C was measured at 1 and 21 days after harvest.

[a] The least significant difference at $P < 0.05$.

[b] NS, *, or ** indicates nonsignificant or significant at $P < 0.05$, and 0.01, respectively.

[c] The values are means of four replicates followed by standard deviations.

There are some conflicting reports on the influence of irradiation on vitamin C content in fresh fruits and vegetables (Thomas 1988). Many have reported irradiation reduced vitamin C content in various fruit and vegetables. Our results suggest that sprouts grown from irradiated alfalfa seeds had higher vitamin C contents than those from nonirradiated seeds. It should be pointed out that most of the earlier studies deal with irradiated plants or sprouts, not seeds.

There are two forms of vitamin C, ascorbic acid and dehydroascorbic acid, both have similar vitamin activity. The FRAP assay measures the ferric reducing ability of sprout extracts. When a ferric-tripyridyltriazine ($Fe3^{+}$-TPTZ) complex is reduced to the ferrous form (Fe^{2+}) by reductants (antioxidants) in sprout extracts, an intense blue color with an absorption maximum at 593 nm develops. The oxidized form of vitamin C, dehydroascorbic acid, cannot reduce $Fe3^{+}$-TPTZ. Therefore, dehydroascorbic acid does not contribute to the total antioxidant power while ascorbic acid does. Besides ascorbic acid, another important group of antioxidants in plant tissues are phenolics. It has been shown that ionizing radiation increased formation of phenolic compounds in a number of plant tissues (Tomas-Barberan and Espin 2001).

A previous study demonstrated that seed irradiation decreased sprout yield at doses above 1 kGy (Rajkowski and Thayer 2001). The increase in antioxidant

and vitamin C of sprouts from irradiated seeds may be a result of stress responses. Irradiation may act as a stressor which induces stress responses as evidenced by the higher respiration rate of sprouts from irradiated seeds. During irradiation, free radicals are generated, and these radicals may then stimulate a mechanism to increase antioxidant power to protect from cellular damage. In doing so, the yield of sprouts may be compromised (Rajkowski and Thayer 2001). The free radicals and subsequent accumulation of radiolytic compounds may also stimulate respiration. Products from the respiration system may be used for damage repair and protection.

Irradiation of seeds did not influence carotenoid content of sprouts at either 1 day or 21 days after harvest (Table 4). During storage, carotenoid content decreased. Seed irradiation did not influence chlorophyll content of sprouts, but chlorophyll content decreased during storage (Table 5). Irradiation of corn seeds increased synthesis of chlorophyll and carotene in seedlings (Vlasyuk and Mar'ina 1970). We did not observe any irradiation-induced increase in chlorophyll or carotenoid probably due to differences in species and/or growing conditions.

TABLE 4.
CAROTENOID CONTENT ($\mu G \cdot G^{-1}$ FRESH WEIGHT) OF SPROUTS DURING STORAGE AT 7C

Radiation dose (kGy)	Storage time (day)		
	1	21	
0	19.6±1.6[c]	13.3±4.2	*[b]
1	20.0±1.3	12.9±2.1	*
2	18.8±1.5	15.7±2.9	NS
3	21.2±6.1	14.3±2.3	NS
$LSD_{0.05}$[a]	6.4	6.6	

The sprouts were raised from seeds irradiated with 0, 1, 2 and 3 kGy gamma radiation. Carotenoids were measured at 1 and 21 days after harvest.

[a] The least significant difference at $P < 0.05$.

[b] NS, *, or ** indicates nonsignificant or significant at $P < 0.05$, and 0.01, respectively.

[c] The values are means of four replicates followed by standard deviations.

TABLE 5.
TOTAL CHLOROPHYLL CONTENT ($\mu G \cdot G^{-1}$ FRESH WEIGHT) OF SPROUTS DURING STORAGE AT 7C

Radiation dose (kGy)	Storage time (day)		
	1	21	
	Total chlorophyll content		
0	57.2±6.6[c]	26.7±16.0	*[b]
1	52.0±5.4	27.2±5.7	*
2	46.0±12.1	31.3±11.3	NS
3	54.8±13.5	24.6±9.4	*
$LSD_{0.05}$[a]	28.6	17.3	

The sprouts were raised from seeds irradiated with 0, 1, 2, and 3 kGy gamma radiation. Chlorophylls were measured at 1 and 14 days after harvest.
[a] The least significant difference at $P < 0.05$.
[b] NS, *, or ** indicates nonsignificant or significant at $P < 0.05$, and 0.01, respectively.
[c] The values are means of four replicates followed by standard deviations.

Compared with those from nonirradiated seeds, sprouts grown from irradiated seeds and stored for 21 days at 6C had significantly ($P<0.05$) higher soluble solids content (3.4, 5.0, 5.0, and 6.0% for 0, 1, 2, and 3 kGy samples, respectively) measured using a reflectometer, indicating that the sprouts from irradiated seeds would probably be sweeter than those from control seeds. A taste panel may be conducted to confirm this finding.

The shoot length of sprouts was not significantly affected by seed irradiation at doses of 1 and 2 kGy, but the root length decreased with higher radiation dose (Table 6). Consequently, the shoot to root ratio increased as dose increased. Short roots and long shoots are desirable quality attributes of sprouts for human consumption (Chen *et al.* 1987). Our results show sprouts grown from irradiated seeds had short roots compared with those from nonirradiated seeds. Physical stresses, such as irradiation, can increase the production of ethylene and ethylene in turn decreases elongation of hypocotyls (Goeschl *et al.* 1966). Exposure of mung bean cuttings to ethylene gas inhibited the growth of roots (Robbins *et al.* 1985). Similarly, exposure to an ethylene releasing agent,

Ethephon, during sprouting also reduced the growth of roots and shoots (Ahmad and Abdullah 1993). Although ethylene production was not measured in our study, other studies have shown that irradiation altered ethylene production of plant tissue (Maxie and Abdel-Kader 1966).

TABLE 6.
LENGTH OF ALFALFA SPROUTS GROWN FROM IRRADIATED SEEDS

Radiation dose (kGy)	Root length (mm)	Shoot length (mm)	Shoot/root
0	35.1 ±8.8[b]	31.4 ±5.5	1.0 ±0.3
1	29.0 ±11.0	30.7 ±4.6	1.2 ±0.5
2	18.9 ±5.1	29.5 ±5.9	1.7 ±0.6
3	14.5 ±3.5	26.4 ±6.8	1.8 ±0.5
$LSD_{0.05}$[a]	3.3	2.5	0.2

Alfalfa sprouts were exposed to 0, 1, 2, and 3 kGy gamma radiation, and then sprouts were grown form the seeds in a commercial sprouting facility. Shoot and root length was measured 1 day after harvest.

[a] The least significant difference at $P < 0.05$.

[b] The values are means followed by standard deviations.

Consumption of fresh vegetables has been associated with lower incidence and lower mortality rates of cancers, and cardio- and cerebrovascular diseases (Machlin 1995). The protection that vegetables provide against these diseases has been attributed to endogenous antioxidants. Antioxidant activity of alfalfa sprouts is one of the highest among the tested vegetables (Cao *et al.* 1996). Vitamin C is one of the antioxidants commonly found in fresh vegetables. Our results showed that alfalfa sprouts grown from irradiated seeds had higher total antioxidant powers and vitamin C measured both immediately after harvest and after 3 weeks storage. The mechanism for the higher antioxidant and vitamin C content needs a further study. Plant pigments, such as chlorophyll and carotenoids contribute to the greenness and yellowness of sprouts. Carotenoids also possess antioxidant powers. Irradiation of seeds did not influence chlorophyll and carotenoid contents of grown sprouts. The pigments in sprouts, however, decreased during storage. Overall our results indicate that irradiation

of seeds for the inactivation of foodborne pathogens does not negatively influence nutritional quality of sprouts.

In summary, seed irradiation increased antioxidant power and vitamin C content of sprouts raised from the irradiated alfalfa seeds. The high vitamin C and antioxidant power were evident even after 21 days storage at 7C. Seed irradiation did not affect chlorophyll or carotenoid content, but decreased root length.

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